

Effects of salinity and desalination on seed germination of six annual weed species

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Abstract: The effects of various salinities and desalination on seed germination of six annual glycophytes (*Artemisia sieversiana*, *A. scoparia*, *Chloris virgata*, *Eragrostis pilosa*, *Chenopodium acuminatum* and *Chenopodium glaucum*) were studied in Horqin Sandy Land, Inner Mongolia, China. NaCl solutions of five concentrations (0 mM, as the control, and 50, 100, 200 and 300 mM) were used for saline stress and desalination treatments. Increasing salinity significantly reduced germination percentages of *A. sieversiana*, *A. scoparia*, *Ch. virgata* and *Ch. acuminatum*, but had no effect on the germination percentages of *E. pilosa*. Lower salinity levels (50 mM) significantly increased germination percentage of *Ch. glaucum*. High salinity might be a precondition for germination after desalination for five of the six species, excepting *E. pilosa* at NaCl concentration of 300 mM in comparison with non-primed seeds. Higher salinity (>200 mM) led to some specific ion toxicity and reduced seed viability of *A. sieversiana*. No specific ion toxicity but an osmotic effect limited the germination of other five species was observed. The final germination percentages (salinity stress and desalination) of the six species showed three variations in comparison with the controls, namely, indiscriminate, stimulation, and reduction. Germination responses to salinity and desalination suggested that the six species were separated into three categories. Three species (*A. sieversiana*, *Ch. virgata* and *Ch. acuminatum*) showed similar germination responses to salinity with those of halophyte, but also showed a lower tolerance limit than most halophytes, although this was not always the case. *A. scoparia* and *Ch. glaucum* exhibited some 'salt stimulation' in seed germination percentages after desalination, whereas *E. pilosa* did not show any obvious response

to salinity. Therefore, salinity usually induces dormancy of seeds with strong germination capacity in fresh water, but has few, or even positive, effects on seeds with strong innate dormancy.

Keywords: annual weed species; germination; salinity; glycophyte; halophyte; Horqin sandy land

Introduction

Salinity is an increasing environmental problem in North Africa, the Middle East, Australia, and other arid regions of the world (Le Houérou 1986; Munns 2002). An excess of salt in the soil has detrimental effects on vegetation in both arid and semi-arid areas (Kennedy et al. 1999; Cayuela et al. 2001). For example, in Horqin Sandy Land, Inner Mongolia, China, 70% of meadow has become saline and sodic (Jiang et al. 2002). In many microhabitats, the continuous intensive evaporation of groundwater gradually deposits salt on the soil surface, where many seeds are distributed; occasionally heavy rainfall can quickly leach salt from the surface, supplying water to the seeds. Thus, for species inhabiting these microhabitats, salinity stress is one of the important environmental factors determining successful seed germination.

Many researchers have studied salinity tolerance as related to germination of halophytes and glycophytes (Ungar 1982, 1991; Khan and Ungar 1996a, 1996b; Keiffer and Ungar 1995; Khan et al. 2000; Tobe et al. 2001; Huang et al. 2003). Most results indicated that many halophyte and glycophyte species show similar responses to high levels of salinity stress, that is, a delay in the initiation of germination and a reduction in the percentage of seeds germinated (Ungar 1962, 1991; Khan and Ungar 1996a, 1996b; Keiffer and Ungar 1995; Tobe et al. 1999). Moreover, high salinity can also cause complete inhibition of seed germination (Ungar 1991). Seeds of such halophyte and glycophyte species generally show optimal germination in fresh water (Macke and Ungar 1971; Waisel and Ovadia 1972; Khan and Ungar 1984; Ungar 1995). However, it might be that halophyte species can germinate at higher salinities than glycophyte species (Woodell 1985; Ungar 1996).

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In Horqin Sandy Land, vegetation rehabilitation by introducing some pioneering halophytic or non-halophytic species, i.e. glycophytes, is an important approach to combat the salinization and desertification of meadows. Annual species showed more aggressive when invading and inhabiting new microhabitats. We selected six annual weed species: *Artemisia sieversiana* Willd., *Artemisia scoparia* Waldstein et Kitaibel., *Chloris virgata* SW., *Eragrostis pilosa* (L.) P. B., *Chenopodium acuminatum* Willd. and *Chenopodium glaucum* L.. Based on previous descriptions of the local flora (Wang et al. 1959, 2004; Qin 2004), these species are all glycophytes that are widely distributed in Horqin Sandy land, and they are important indicators of degradation of meadow vegetation. Germination is assumed to be the key life-cycle stage for annual species but published data on the germination of these annual weed species are limited (Li et al. 2006). In particular, no data are available to quantify seed germination under saline conditions. In this study, we examined the effects of salinity on germination of six annual species. We also discuss the salinity tolerance of glycophytes during seed germination and their germination capacity after desalination.

Materials and methods

Mature seeds of each species were collected directly from plants in the western region of Horqin Sandy Land from September to November in 2004. Seeds were cleaned after collection and then placed in paper bag in darkness at the laboratory. All experiments were carried out in April 2006.

For each species, groups of 4×50 viable seeds were sown onto two layers of filter paper in 90-mm Petri dishes. The filter paper was moistened with NaCl solutions of different concentrations (0 mM as the control, and 50, 100, 200 and 300 mM). Each dish was covered with a lid and transferred to a germination chamber with fluorescent lamps that provided visible radiation of $112.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, over a 15 hour day at 28°C and $0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ over a 9-hour night at 16°C , approximating the average daily maximum and minimum temperatures recorded in soil during the growing season from May to August. The number of germinated seeds was counted and germinated seeds were removed daily for nine days. Non-germinated seeds were desalted completely and transferred to new Petri dishes in distilled water to test their germination capacity following saline stress. The number of germinated seeds was then counted and germinated seeds were removed for another nine days. Seeds were considered germinated once the radicle had emerged. The viability of non-germinated seeds after desalination was tested by TTC test (triphenyltetrazolium chloride).

The results are presented as seed germination percentages and viable non-germination seed percentages in total seeds under saline stress and desalination. Germination percentages and viable non-germination percentages were compared using a one-way ANOVA to evaluate the effects of salinity and desalination on seed germination and viability. Data were normalized by square-root transformation prior to statistical analyses. Where significant differences were found, means were separated using

the LSD test at 95% confidence. All statistical analyses were performed with the SPSS statistical package (version 10.0, 1999).

Results

Following nine days of exposure to saline conditions, seed germination percentages were determined (Fig. 1). For *A. sieversiana*, *A. scoparia* and *Ch. acuminatum*, seed germination percentages were significantly reduced by NaCl concentrations of 50 mM in comparison with control values ($p=0.000$). For *Ch. glaucum*, seed germination percentage was significantly increased in 50 mM NaCl in comparison with control values ($p=0.000$). Seed germination percentages of *Ch. glaucum* in 100 mM did not show any difference from the control value. For the four species mentioned above, seed germination was totally suppressed at NaCl concentration of 200 mM. For *Ch. virgata*, seed germination percentage was significantly reduced in 100 mM NaCl, and totally suppressed at 300 mM NaCl ($p=0.000$). For *E. pilosa*, seed germination decreased significantly at 300 mM NaCl in comparison with the control ($p=0.000$).

Following nine days of exposure to distilled water after desalination, seed germination percentages were determined (Fig. 1). For *A. sieversiana*, *A. scoparia*, *Ch. virgata* and *Ch. acuminatum*, seed germination percentages after desalination were increased significantly, compared with germination levels at low to moderate salinity ($p=0.000$). For *E. pilosa*, only 7% of total seeds originally placed in 300 mM NaCl germinated ($p=0.000$). For *Ch. glaucum*, the highest germination percentage after desalination came from seeds originally placed in 100 mM, the second in 300 mM, the third in 200 mM, and the lowest in 50 mM, all of which differed significantly from the control value ($p=0.000$).

Seed germination percentages of saline stress and desalinization showed different responses for the six species (Fig. 1). For *A. sieversiana*, seed germination percentages did not differ between the control, 50 and 100 mM, but significantly decreased in 200 and 300 mM ($p=0.000$). For *Ch. virgata* and *Ch. acuminatum*, the highest germination value under saline stress and desalinization did not show any difference with the control values, but the lowest germination value was significantly lower than that of the control values ($p<0.05$). For *A. scoparia* and *Ch. glaucum*, the highest germination value under saline stress and desalinization was significantly higher than that of the control values ($p<0.05$) but the lowest values did not show any difference with the control values. For *E. pilosa*, the highest germination value was 36% in the control, and the lowest value significantly decreased to 24.5% in 300 mM.

Viable seed percentages including germination and viable non-germination were determined (Fig. 1). For *A. scoparia*, *Ch. virgata*, *E. pilosa*, *Ch. acuminatum* and *Ch. glaucum*, viable seed percentages did not show any significant difference between the control and the different treatments of saline stress and desalinization. For *A. sieversiana*, viable seed percentages did not show any significant difference in the control, 50 and 100 mM, but viable seed percentages in 200 and 300 mM were significantly

lower than the control value ($p < 0.05$).

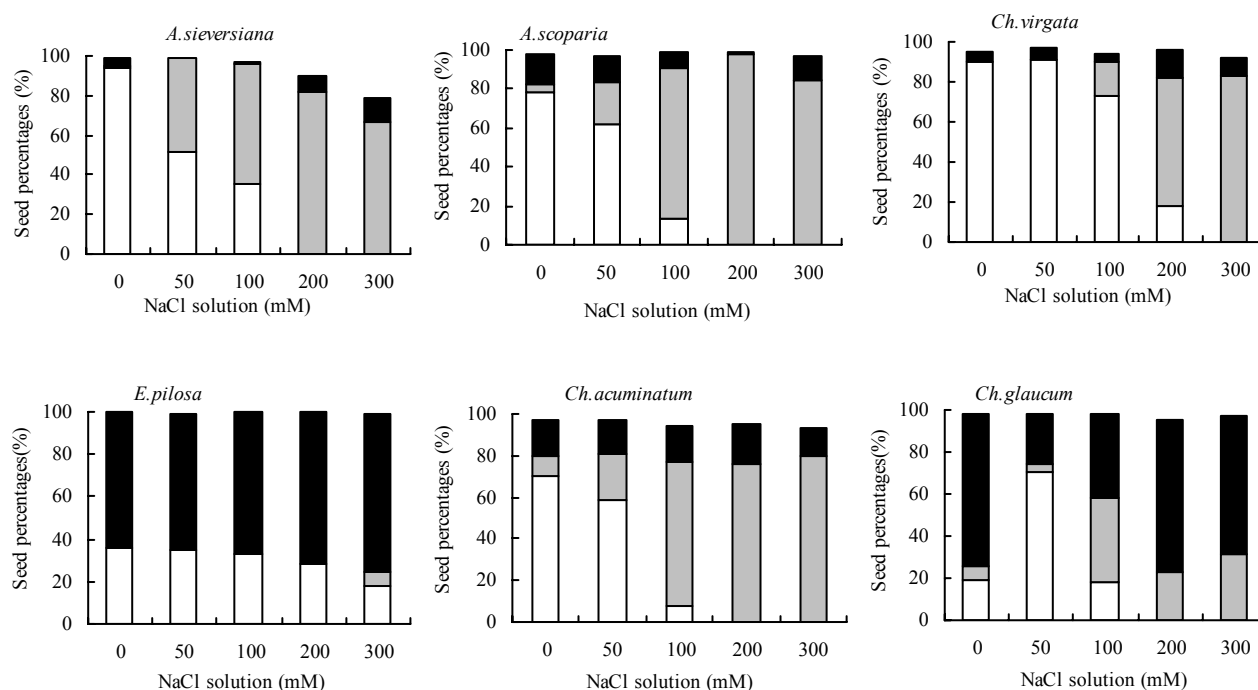


Fig. 1 Germination percentages and viable non-germination percentages in total seeds of six annual glycophytes in Horqin Sandy Land

(□)= Germination percentages of seeds exposure to different saline stress for 9 days; (■)= Germination percentages of seeds exposure to distilled water for 9 days after desalination; (■)= Viable non-germination seed percentages after saline stress and desalination.

Discussion

The seeds of six annual glycophytes tested here showed three different germination responses to different salinity concentrations. Seed germination decreased significantly with increasing salinity for *A. sieversiana*, *A. scoparia*, *Ch. virgata* and *Ch. acuminatum*. Similar trends have been found in many halophytes (Khan and Ungar 1996a, 1996b; Khan et al. 1998; Katembe et al. 1998; Ishikawa and Kachi 2000; Khan and Ungar 2001; Khan et al. 2003). Moderate (50–200 mM) but higher saline concentration (300 mM) did not significantly inhibit seed germination of *E. pilosa*, whereas lower salinity (50 mM) significantly increased seed germination percentage of *Ch. glaucum*.

Previous literature reported that halophytes vary in their ability to tolerate salinity during germination [e.g. *Haloxylon ammodendron*, 1400 mM NaCl (Huang et al. 2003), *Arthrocnemum indicum*, 800–1000 mM (Khan et al. 1998), *Tamarix pentandra*, 850 mM (Ungar 1967), *Artemisia fukudo*, and *Artemisia stelleriana*, 500 mM (Ishikawa & Kachi 2000), *Atriplex prostrata*, 300 mM (Khan et al. 2003), *Halopyrum mueronatum*, 300 mM (Khan & Ungar 2001), *Atriplex patula*, 170 mM (Ungar, 1996; Katembe et al. 1998), *Zygophyllum simplex*, 125 mM (Khan & Ungar 1996a)]. For *A. sieversiana*, *A. scoparia*, *Ch. acuminatum* in Horqin Sandy Land, the salinity tolerance limit for seed germination was 200 mM. This suggests that halophyte species are

better able to germinate under conditions of higher salinity, compared with many glycophyte species (Ungar, 1996); however, this is not always the case. For *Ch. virgata*, the salinity tolerance limit for seed germination was 300 mM, indicating that it has a higher salt tolerance than do three other species (*A. sieversiana*, *A. scoparia*, *Ch. acuminatum*). For *E. pilosa*, 24.5% of seeds germinated following exposure to 300 mM NaCl, which indicated that this species had higher salt tolerance than did other four species (*A. sieversiana*, *A. scoparia*, *Ch. acuminatum* and *Ch. virgata*). The fact that germination of *Ch. glaucum* was suppressed in 200 mM NaCl, but the seeds exhibited significant “salt stimulation” in 50 mM NaCl is an interesting phenomena that should be investigated further.

Many studies on the seed germination capacity after desalination have been reported (Heydecker et al. 1973; Osborne et al. 1981; Ungar 1995; Khan and Ungar 1996b, 1997; Huang et al. 2003). In our study, *A. sieversiana*, *A. scoparia*, *Ch. virgata* and *Ch. acuminatum* showed similar germination responses (i.e. the higher the NaCl concentrations to which seeds were exposed, the higher the germination percentages after desalination). Similar germination characteristics were found in seeds of *Haloxylon recurvum* (Khan & Ungar 1996b) and *H. ammodendron* (Huang et al. 2003). Seeds of *Ch. glaucum* and *E. pilosa* different germination responses after desalination from other four species (*A. sieversiana*, *A. scoparia*, *Ch. virgata* and *Ch. acuminatum*). But salinity of 100–300 mM for *Ch. glaucum* and 300 mM for *E. pilosa* might act as preconditioning treatment and could increase

germination percentage in comparison with non-primed seeds (Heydecker et al. 1973; Osborne et al. 1981).

There were two different responses for the highest germination values under salinity and desalination of six species [i.e. indiscrimination (*A. sieversiana*, *Ch. virgata*, *E. pilosa* and *Ch. acuminatum*) versus stimulation (*A. scoparia* and *Ch. glaucum*)]; Similarly, the lowest germination percentages also showed two responses [i.e. indiscrimination (*A. scoparia* and *Ch. glaucum*) versus reduction (*A. sieversiana*, *Ch. virgata*, *E. pilosa* and *Ch. acuminatum*)]. Here, we consulted the Woodell (1985) halophyte classification system and categorized the six annual glycophytes seeds as being three types: Type 1 (*A. sieversiana*, *Ch. virgata* and *Ch. acuminatum*): seeds germination was usually inhibited by salinity, and seed germination percentage after desalination was relatively high. Type 2 (*A. scoparia* and *Ch. glaucum*): seeds germination was usually inhibited by higher salinity levels, but salt stimulation after desalination was observed. Type 3 (*E. pilosa*): seed germination was not usually inhibited by salinity and germination percentages after desalination (if it occurred at all) was relatively low.

Based on percentages of viable seeds after treatments of saline stress and desalination, there were two results for six species: *A. scoparia*, *Ch. virgata*, *E. pilosa*, *Ch. acuminatum* and *Ch. glaucum* were characterized by indiscrimination, while *A. sieversiana* was characterized by reduction. We therefore conclude that there is no specific ion toxicity and that an osmotic effect limits the germination of five species but not *A. sieversiana*. Viable seed percentages of *A. sieversiana* declined in 200 and 300 mM by 9% and 20.5%, respectively, in comparison with control value. We deduced that higher salinity stress perhaps leads to some specific ion toxicity and reduces seed viability of *A. sieversiana*.

The results presented here indicate that six annual glycophytes vary greatly in their germination responses when exposed to varying salinities and after desalination. Four species (*A. sieversiana*, *A. scoparia*, *Ch. acuminatum* and *Ch. glaucum*) the six annual glycophytes exhibited lower salinity tolerance (about 200 mM) for germination than the most tolerant halophyte species (> 300 mM). However, the tolerance of these glycophyte species is higher than that of some halophyte species (*A. patula*: about 170 mM and *Z. simplex*: about 125mM) remains unexplained (Ungar 1996; Khan & Ungar 1996a; Katembe et al., 1998). This is an interesting phenomenon that requires further study.

Germination stimulation for *A. scoparia* and *Ch. glaucum* by salinity exposure, and the lack of any obvious inhibition of germination of *E. pilosa* seeds following salinity exposure were also interesting results. Each of these three annual species has a relatively broad geographic distribution and shows strong resistance to disturbance in semiarid regions (Wang et al. 1959, 2004; Qin 2004). One important reason for this is that their fresh seeds have strong innate-dormancy. A fraction (18%–50%) of seeds will not germinate even after dry storage for up to a year (Li et al., 2006). In this study, these three species also showed individual germination responses to salinity. The strong innate dormancy of seeds in 50 mM was obviously broken for *Ch. glaucum*. Seed germination percentages were significantly increased following saline stress in 50 and 100 mM for *Ch. glaucum* and in 200 mM for *A. sco-*

paria (both with subsequent desalination). Salinity stress (except 300 mM) had no significant effects on *E. pilosa* seed germination or their innate dormancy. We suggest that the different seed germination responses to salinity between these and other annual species should be investigated further, particularly in view of the increasing aridity of many areas of the world.

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